EXHIBIT 2

Helicobacter pylori
Physiology and Genetics
Edited by Harry L.T. Mobley, George L. Mendz, and Stuart L. Hazell
ASM PRESS

35. Lipopolysaccharide Lewis Antigens

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Helicobacter pylori
Neisseria gonorrhoeae

As determined by serological techniques, the O-antigen of lipopolysaccharide (LPS) of more than 80% of *Helicobacter pylori* strains tested worldwide express Lewis blood group antigens (36, 60, 71). This percentage possibly represents an underestimation; it was demonstrated that some *H. pylori* strains do not react with anti-Lewis x (Le^x) monoclonal antibodies (MAbs) while structurally they were shown to express Le^x (39). Thus, Lewis antigen expression in *H. pylori* is highly conserved. This restricted diversity in O-antigen structure is striking, and the question arises whether *H. pylori* Lewis antigens play a role in pathogenesis. An analogous situation is found in *Neisseria gonorrhoeae*, where conserved LPS O-antigen epitopes directly interact with the host via ligand-lectin binding (35).

H. pylori
Haemophilus influenzae
Campylobacter jejuni

There are additional reasons why *H. pylori* LPS Lewis antigens are thought to play a role in pathogenesis beyond merely providing length to the LPS (although length itself already contributes to virulence) (7). (i) *H. pylori* LPS displays phase variation, defined as the high frequency of reversible change of LPS phenotype (2, 5, 68, 69). In other bacteria (*Neisseria* spp. and *Haemophilus influenzae*), phase variation of LPS is crucial to virulence (37, 65). (ii) *H. pylori* LPS displays molecular mimicry with the host (4). Gastric human epithelial cells also express $Le^{x/y}$ blood group antigens. The expression by microorganisms of surface structures similar to those found in the host is called molecular mimicry. Examples of other pathogens displaying molecular mimicry are *Campylobacter jejuni* and *Neisseria* spp. (33). The role of mimicry in pathogenesis can be twofold. (a) *H. pylori* mimicry is pathogenic. Infection might break tolerance to the shared epitopes and induce autoantibodies. Bound antibodies may induce tissue damage, for instance, by fixing complement. (b) Molecular mimicry might provide immune escape by preventing the formation of antibodies directed to the epitopes shared by self and microorganism; the lack of response to a surface-located antigen might

blood group...
Table 2. LPS phase variants of strains...
Table 3. Reactivity of monoclonal antibodies with...

contribute to persistence of infection. (iii) *H. pylori* Lewis antigens might interact with host lectins. Several host lectins are known to interact with host Lewis antigens (22, 42); the same lectins may interact with *H. pylori* Lewis antigens. Such interaction may have biological consequences such as bacterial adhesion, colonization, and cytokine induction.

In this chapter, we will discuss phase variation of *H. pylori* LPS, including LPS biosynthesis and genetics; the biological significance of Lewis antigen mimicry; and the role of Lewis antigens in interactions of *H. pylori* with host lectins. H. pylori

Phase Variation in *H. pylori* LPS

The structures of LPS isolated from a variety of *H. pylori* strains have been determined chemically. The overall architecture of *H. pylori* LPS is similar to that of LPS of other gram-negative pathogens. The lipid A moiety is connected to the oligosaccharide core region that in turn is connected to the O-antigen (or Lewis antigen). In many strains, the O-antigen consists of Le^x and/or Le^y (Table 1), but other blood group antigens (H type 1, Le^a, Le^b, nonfucosylated polylactosamine [=i-antigen], sialyl Lewis x, blood group A) have also been found (10, 11, 46, 47, 49). Strains expressing H type 2 have not been identified. Often, strains express more than one Lewis antigen (Table 2). For example, strain NCTC 11637 (ATCC 43504) expresses polymeric Le^x with n up to 8 or 9 that is substituted terminally in nonstoichiometric amounts with Le^y or H type 1. H. pylori

Phase Variation

Phase variation is defined as the random switching of LPS phenotype at frequencies that are much higher (sometimes >1%) than classical mutation rates. This process results in reversible loss and gain of certain LPS epitopes and results in a bacterial population that is heterogeneous with regard to LPS expression. Phase variation contributes to virulence by generating heterogeneity; certain environmental or host pressures select those bacteria that express the best adapted phenotype. An example is LPS sialylation in *Neisseria* spp. While nonsialylated bacteria are adherent and invasive, they are sensitive to the lytic action of serum; in contrast, sialylated bacteria adhere less well but are more resistant to serum (65). Phase variation allows outgrowth of nonsialylated bacteria during adhesion or invasion and of bacteria expressing sialylated LPS upon contact with serum.

Phase variation can be detected by colony-blotting with MAbs specific for LPS (5). An example is given in Fig. 1 where an *H. pylori* strain was probed with a MAb specific for H type 1. Three types of colonies are present: first, those that are completely reactive (dark colonies); the bacteria forming this colony originate from a single bacterial cell expressing H type 1, with no switching off to the H type 1-negative phenotype occurring during multiplication. Likewise, nonreactive colonies originate from a bacterial cell with a switched-off phenotype. Colonies with a dark sector originate from a cell with a switched-off phenotype that switched on during multiplication (often on more than one independent event per colony); clonal outgrowth of a switched-on variant gives rise to the sectors observed. By colony-blotting, many LPS phase H. pylori

variants were isolated from a single strain (NCTC 11637) (see Table 2).

Subsequently, variants were serotyped in enzyme-linked immunosorbent assay and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (Fig. 2, Table 2 and 3). The frequency of phase variation is in the range of 0.5 to 1%, but the frequency of switching on is not necessarily the same as switching off: the switch frequency of NCTC 11637 to variant 1b is in the 0.5 to 1% range, but the switch-back frequency to parent phenotype is only 0.07% (5). Phase variation is not restricted to laboratory strains; it also occurs in other strains including clinical isolates.

Molecular Mechanisms of LPS Phase Variation

The sequencing of the genome of two *H. pylori* strains has identified many LPS-related genes, including several glycosyltransferases potentially involved in phase variation (1, 12, 59, 62). Two similar but not identical α3-fucosyltransferse (α3-*fucT*) genes have been identified both in strain 26695 (HP0379 and HP0651) and in strain J99 (JHP1002 and JHP0596). Functional studies with the cloned and expressed gene products show that both FucT enzymes encoded by these two genes are able to form Le^x from lactosamine acceptors (31, 44). However, insertional mutagenesis studies have shown that they differ in fine-specificity (2). The HP0379-encoded α3-FucT has a preference for internal GlcNAc residues (i.e., not located at the nonreducing terminus) and yields polymeric Le^x, while HP0651-encoded α3FucT has a preference for terminal GlcNAc residues and forms mono/oligomeric Le^x. The HP0379-encoded α3-FucT can also function as an α4-FucT and can therefore also form $Le^{a/b}$ (3, 57).

HP0379
HP0651
JHP1002
JHP0596
H. pylori

HP0093/94 (JHP0086) is an α2-*fucT*; the gene product is required for biosynthesis of both Le^y and H type 1 (see below) (3, 67, 69). H type 2 epitopes do not occur in *H. pylori* LPS, and knocking out both α3-*fucT* genes in a strain that expresses $Le^{x/y}$ yields LPS that expresses i-antigen but no H type 2 (2). Thus, α3-fucosylation precedes α2-fucosylation. This was confirmed in enzyme assays with cloned α2FucT that forms Le^y from synthetic Le^x but not H type 2 from Galβ1→4GlcNAc (67). In contrast, this enzyme is able to form H type 1 with a Galβ1→3GlcNac acceptor.

HP0093
JHP0086
H. pylori

Sequencing of α2- and both α3-*fucT* genes revealed that they all carry long poly-C stretches close to the 5′ end of the gene. C-tracts are also present in LPS genes of *Neisseria* spp. and are a well-characterized cause of LPS phase variation (37). On replication, DNA slippage (slipped-strand mispairing) in C-tracts may give rise to daughter DNA that is either one C shorter or longer; this can occur at very high (1%) frequencies. The result is a high-frequency, reversible frameshifting. The consequence is a rapid on-off switching of enzyme activity. When a C-tract is present in the parent strain that to leads to a full-length, active gene product in the C + 1 or C − 1 daughters, the frameshifting will lead either to the production of nonsense polypeptides that

HP0651
HP0379
H. pylori

have no or little enzyme activity or, due to the occurrence of early stop codons, to a truncated inactive gene product. The molecular basis of phase variation in *H. pylori* was determined by sequencing the C-tracts in the α3-*fucT* genes of the parent strain (NCTC 11637) and in the phase variants (Table 3) (2). In the NCTC 11637 HP0651 is "off" due to the presence of a C9 tract; HP0379 is "on" in this strain (C10).

Phase variation from Le^x to i-Ag and back to Le^x

HP0651
HP0379

In the phase variant expressing i-Ag plus H type 1 (variant K4.1), both HP0651 (C9) and HP0379 (C11) are off; this explains the lack of Le^x and the biosynthesis of nonfucosylated polylactosamine (= i-antigen) in strain K4.1. In addition, K4.1 expresses H type I due to the presence of an active α2FucT. In strain K5.1, the Le^x-positive switch-back variant isolated from K4.1, HP0379 is "on" again (C10). Thus, phase variation from Le^x to i-Ag and back to Le^x can be understood at the molecular level through reversible length changes in the C-tract of α3-*fucT* gene HP0379, that is, from C10 to C11 and back to C10. A HP0379/HP0651-double knockout of strain 4187E (4187E-KO379/651) expresses a serotype identical to that of strain K4.1 (i.e., i-antigen and H type 1). Clinical isolate J233 expresses H type 1 plus i-Ag both as determined by structural chemistry (47) and by serology, and in that strain also both α3-*fucT* genes are off. We conclude that LPS serotype is determined by the on-off status of α3-*fucT*.

Phase variation from Le^x to Le^x plus Le^y

HP0379
HP0651

While strain NCTC 11637 expresses polymeric Le^x, H type 1, and a little Le^y, phase variant 1c strongly expresses both Le^x and Le^y. C-tract analysis shows that both HP0379 and HP0651 are "on" in strain 1c. Knockout studies in strain 4187E also show that the presence of an intact HP0651 is associated with a stronger Le^y expression and with reactivity with Mab 6H3 that recognizes monomeric Le^x. We conclude that HP0651 FucT preferentially fucosylates GlcNAc at the nonreducing terminus, thus forming an efficient acceptor for α2-FucT to form Le^y. In contrast, HP0379 α3-FucT would prefer internal GlcNAc, thus forming polymeric Le^x from the inside out, a structure that is evidently a less efficient acceptor. Consequently, as compared to variant 1c, less Le^y is formed in the parent strain.

Phase variation from Le^x to Le^y

HP0379
H. pylori

Variant 1b has a truncated LPS (Fig. 2) that strongly expresses Le^y (Table 2); this serotype is similar to that of strains MO19 and O6. Enzymatic analysis showed that this variant lacks GlcNAcT activity (5). The serotype of this strain can be explained by the following model. Likely there are two GlcNAcT enzymes, one that recognizes the core and adds the first GlcNAc and a second one that recognizes Gal and thus is responsible for chain elongation. Likely, the lack of GlcNAcT activity in variant 1b signifies lack of the second, elongating enzyme. Thus, first the core plus a single GlcNAc is formed in this variant. HP0379 is "on" in variant 1b, so that terminal Le^x is formed;

α2-FucT then forms Le^y. Although GlcNAcT genes have been identified in other species (13), they do not show significant homology with *H. pylori* open reading frames.

Phase variation forming Le^a

Variant 3a expresses polymeric Le^x plus Le^a (3). Hence, compared to NCTC 11637, this variant has lost both Le^y and H type 1. This can be explained by phase variation in α2-*fucT*, and indeed insertional inactivation of this gene in NCTC 11637 yields a mutant with a serotype indistinguishable from that of strain 3a (3). The α2-*fucT* gene also contains a C-tract and hence phase variation occurs along the lines sketched above for a α3-*fucT*. However, a second mechanism for phase variation was observed in the α2-*fucT* gene, namely a sequence (AAAAAAG) that allows mRNA slippage at the translational level (69). The result of this slippage is a −1 frameshift. The mechanisms involved are as follows: there are two anticodons for lysine, UUU and CUU. However, from the whole genome sequence it is known that *H. pylori* codes only for a $tRNA^{Lys}$ with the UUU anticodon while $tRNA^{Lys}$ with the CUU anticodon is missing. Hence, when AAG is encountered in the mRNA of α2*fucT*, the loaded $tRNA^{Lys}$ (UUU) slips one base back to allow the stronger interaction with AAA. This second mechanism may therefore compensate for 31 frameshifting due to C-tracts. These two mechanisms operate in the genome strain 26695. While this strain expresses Le^y (46), its α2-*fucT* gene is frameshifted (+1) due to the C-tract (62) and theoretically would yield an inactive α2FucT. However, presence of the translational −1 frameshift cassette AAAAAAG causes a −1 shift in the reading frame, an active enzyme to be formed and Le^y synthesis to take place. The mechanism of −1 slippage has been well investigated for the *dnaX* gene of *Escherichia coli* (29, 63).

H. pylori
Escherichia coli

Other phase variants

Variant H11 expresses Le^x, Le^y, but no H type 1; hence, phase variation has to take place in the gene coding for β3-GalT (3). Variant D1.1 expresses a truncated LPS and does not react with any anti-Lewis MAb. This variant arose through phase variation from K4.1 through subsequent loss of the elongating GlcNacT (5). An sLe^x-expressing variant of P466 was isolated and characterized (46); *neuB* (HP0178), a gene required for biosynthesis of sialyl-Le^x, contains a C6-tract in strains 26695 and J99.

HP0178

Biological Role of LPS Phase Variation

Is phase variation relevant in vivo? We isolated 30 *H. pylori* colonies from a single patient and found that 20% of the colonies expressed $Le^{x/y}$, while 80% expressed the i-Ag (8). By molecular typing, combined with C-tract sequencing, it was demonstrated that they are phase variants of the same strain. Thus, LPS phase variation contributes to strain diversity in vivo. The data shown above demonstrate that many of the currently known *H. pylori* LPS serotypes can be isolated as phase variants from a single strain (Table 2), and hence, theoretically any strain can express almost any LPS phenotype. Which factors determine the actual serotype expressed by a strain isolated from a clinical sample, or the distribution of serotypes

H. pylori

of multiple isolates obtained from a single patient? At present no single environmental or host factor has been identified that causes a change in LPS phenotype through selection of LPS phase variants. Prolonged growth of bacteria on solid agar leads to reversible loss of O-antigen (51), but whether phase variation is involved is not known.

The Biological Role of *H. pylori* Lewis Antigen Mimicry

H. pylori Mimicry Is Pathogenic

C. jejuni
H. pylori
Schistosoma mansoni

Mimicry can contribute to pathogenesis during infection due to *C. jejuni* (50). LPS of this bacterium expresses ganglioside structures similar to those occurring in nerve tissue. Upon infection, antiganglioside antibodies are formed that cause an autoimmune attack of peripheral nerves followed in some cases by paralysis (Guillain-Barré syndrome). Likewise, *H. pylori* LPS might induce anti-$Le^{x/y}$ antibodies that bind to the bacteria but also to the gastric epithelial cells; when followed by complement fixation this may lead to tissue injury (4). Indeed, immunization of mice with *H. pylori* induces anti-$Le^{x/y}$ MAbs that cross-react with gastric epithelium, in particular with gastric H^+,K^+-ATPase, the proton pump that is localized in the parietal cell canaliculi (Fig. 3) (4, 6). *H. pylori* infection in mice also induces autoantibodies that bind to parietal cells and that can be absorbed with synthetic Lewis antigen (34). Thus, in the murine system, *H. pylori* induces autoantibodies through mimicry. Moreover, high concentrations of circulating anti-Le^y MAbs may cause gastric damage (52). It was already known that *H. pylori* infection in humans also induces autoantibodies that recognize gastric parietal cells (27, 28, 52, 53), and in analogy with the *H. pylori* infection in mice, it was thought that those human autoantibodies also arose through mimicry. Indeed, in patient sera, high titers of antibodies to *H. pylori* LPS are found (6). However, the epitope-specificity of human anti-*H. pylori* LPS remains enigmatic; in an initial study, anti-Le^x antibodies were found in only a few patients' sera (6). However, in a larger survey comprising more than 100 patients, *H. pylori* infection was not found to induce anti-$Le^{x/y}$ antibodies in humans (19). In fact, anti-$Le^{x/y}$ antibodies occur naturally in sera from persons not infected by *H. pylori* (18). One exception might be nonsecretors (persons who do not express Le^b in gastric mucosa) where low affinity, *H. pylori*-associated anti-$Le^{x/y}$ antibodies were detected in serum (40). The question remains as to what epitopes of *H. pylori* LPS human antibodies are directed. Data have been presented that show that fucose is not part of the epitope recognized by human anti-*H. pylori* LPS antibodies, but the nature of this epitope remains elusive (75). Finally, antigastric autoantibodies present in sera of *H. pylori*-infected patients are directed to gastric parietal canaliculi, but absorption with *H. pylori* does not diminish autoantibody reactivity (26). This shows that the *H. pylori*-associated antigastric autoantibodies are not due to mimicry; further studies showed them to be directed to peptide epitopes of gastric H^+,K^+-ATPase (19). Thus, present data suggest that *H. pylori* $Le^{x/y}$ antigens do not induce autoantibodies in infected human patients. Humans are not per se unable to form anti-Le^x antibodies. Patients infected with *Schistosoma mansoni*, a tropical parasite that also expresses Le^x, develop serum antibodies to Le^x that are cytotoxic for Le^x (=CD15)-carrying leukocytes (54, 64). Why *H. pylori* does not induce serum anti-Le^x antibodies is not known. However, it cannot be excluded that *H. pylori* induces anti-$Le^{x/y}$ antibodies locally that bind directly

to gastric mucosal epitopes, so that they do not appear in serum.

Lewis Antigen Mimicry and Immune Evasion

H. pylori
H. mustelae

By analogy to the ABO blood group antigens, one might predict that a host that expresses Le^x would be expected to form anti-Le^y but not anti-Le^x antibodies. Hence, a Le^x-positive *H. pylori* strain that infects an Le^x-positive host would escape immune attack and be able to persist, while an Le^y-positive strain would not escape and would be eradicated. Experimental infection in rhesus monkeys confirms this concept: an *H. pylori* strain isolated from Le^y-positive animals (in gastric mucosa) expresses more Le^y than Le^x; the same strain expresses more Le^x than Le^y when isolated after colonization of Le^x positive animals (72). Thus, the expression of *H. pylori* $Le^{x/y}$ epitopes depends on the host. It is conceivable that in vivo outgrowth of Le^y-expressing *H. pylori* variants is favored because variants expressing Le^x are suppressed in Le^y-positive hosts that form anti-Le^x but not anti-Le^y antibodies. However, whether the two variants isolated are phase variants was not investigated, nor was it shown that the animals formed serum antibodies to $Le^{x/y}$. Studies in humans gave far less consistent results and, in two out of three studies, no correlation between the Lewis phenotypes of host and pathogen was found (36, 61, 74). In addition, strains expressing Le^x and strains expressing Le^y can be isolated from a single patient, an additional argument against adaptation based on Lewis antigens (73). Finally, selection and outgrowth of *H. pylori* $Le^{x/y}$ LPS variants would be driven by anti-$Le^{x/y}$ antibodies, and these are not found in infected patients (19). Despite these objections with regard to a role for Lewis antigen mimicry in immune evasion, it remains striking that ferrets, whose gastric epithelium is blood group A-positive, are colonized by a helicobacter species (*H. mustelae*) that also expresses blood group A (21, 48). It is also striking that *H. pylori* strains isolated from Chinese patients more often express Le^a or Le^b as compared to strains isolated in Western countries (76), while Chinese themselves also express the Le^{ab}-positive phenotype more often as compared to Caucasians.

H. pylori Lewis Antigens as Adhesins

H. pylori

Several host lectins are already known to interact with host Lewis antigens. For example, selectins bind to Le^x and, in particular, sLe^x (22, 42). Furthermore, several other C-type (calcium-dependent) lectins are known to interact specifically with mannose. Examples are mannose-binding protein, surfactant protein D, and macrophage mannose receptor (70). Mannose and fucose share the presence of two adjacent, equatorial OH– groups that are required for calcium-dependent interaction with this group of lectins. Hence, it is likely that fucosylated *H. pylori* LPS interact with C-type host lectins (see below).

H. pylori

Studies on the biological role of *H. pylori* Lewis antigens have largely taken place through insertional mutagenesis of LPS biosynthesis genes. The expression of $Le^{x/y}$ proved to be crucial for in vivo colonization of mice: the gene encoding β1,4 GalT was inactivated in strain SS-1

(expresses $Le^{x/y}$) (43). The mutant expresses a shorter LPS devoid of Lewis antigens and, in contrast to the parent strain, colonizes mice less well. However, the lack of colonization does not prove that Lewis antigens per se are essential: from other gram-negative pathogens it is known that shortening of LPS will lead to a decrease in virulence. Strains with a shorter LPS are simply more sensitive to the lytic action of serum or are more easily phagocytosed.

A double knockout was created in strain 4187E in which both α3-*fucT* genes were inactivated (4187E KO0379/0651) (see Table 3). This mutant expresses a long polylactosamine chain (i-antigen) and H type 1. The parent strain ($Le^{x/y}$ positive) colonizes mice well, but the mutant does not, which demonstrates that $Le^{x/y}$ antigens are essential for colonization (45). However, in another study, an α3-*fucT* double knockout colonized as well as its parent (16).

HP0360
HP0043
H. pylori

Recent data suggest that Le^x plays a role in adhesion. A MAb specific for *H. pylori* LPS inhibits adhesion of bacteria to gastric epithelial cells (56); this MAb is specific for Le^x (9). Further data on the role of Lewis x in adhesion were again obtained from knockout studies. Strains with a mutation in *galE* (HP0360, UDP-galactose-4-epimerase) yield a truncated LPS (24, 41) that lacks galactose (24). A strain knocked out in gene *rfbM* (HP0043, GDP-mannose pyrophosphorylase) yields a fucose-lacking LPS that expresses the i-antigen (24). *rfbM* is involved in biosynthesis of GDP-mannose, a precursor of GDP-fucose, which is the fucosyl donor of both α2- and α3-FucT. Both the *galE* and the *rfbM* mutant did not adhere to gastric sections, while the parent (strain NCTC 11637, $Le^{x/y}$ positive) adhered well (24). Infection studies with a *galE* mutant showed it to colonize less well than its parent (51a). In addition, synthetic Le^x coupled to 1 μm-sized polystyrene beads bound to human gastric epithelial cells (24). Clinical studies also suggest a role for $Le^{x/y}$ in adhesion; studies in gastritis patients demonstrated that *H. pylori* strains that expressed $Le^{x/y}$ strongly cause a higher colonization density than strains that express $Le^{x/y}$ weakly (36). In addition, a strong Lewis antigen expression of the infecting strain was associated with an increased influx of polymorphonuclear leukocytes (36). These data suggest that Le^x mediates colonization through adhesion, predict the existence of gastric Le^x-binding lectins, and suggest an association between adhesion and inflammation. Indeed, Le^x-binding lectins of 16 to 29 kDa (17) and 100 kDa (23) are found in the AGS gastric epithelial cell line; the identity of these proteins is unknown, but the presence of low molecular weight lectins (galectins) in the stomach has been reported (55). Other studies have shown that surfactant protein D, a C-type lectin belonging to the innate defense system and expressed in the stomach (30), is able to bind *H. pylori* LPS (25); it is unknown which moiety of the LPS is recognized. Thus, a role for LPS/$Le^{x/y}$ in adherence seems likely, but this role is not absolute. $Le^{x/y}$-negative mutants adhered as strongly as their $Le^{x/y}$-positive parents when the strain expresses the Le^b-binding lectin BabA and when the host expresses Le^b (14). In addition, $Le^{x/y}$-negative strains colonize human hosts well (58). Thus, an $Le^{x/y}$-lectin interaction may contribute to adhesion only for *H. pylori* strains that do not express BabA or for strains that colonize nonsecretors. Likewise, it is known that *H. pylori* can colonize mice, even when they do not express Le^b (34), the counter ligand of BabA (15, 38); colonization of mice might require the presence of Le^x-binding lectins in the gastric mucosa. Phase

variation might fulfill a biological role by allowing detachment of bacteria not expressing $Le^{x/y}$ and hence transmission to another host; subsequently, switch-back variants expressing $Le^{x/y}$ adhere and colonize a new host. Interestingly, variants that do not bind surfactant protein D have been isolated but colonization studies have not been performed with these strains (66).

Adhesion of *H. pylori* has clinical relevance: strains from ulcer patients more often express BabA compared to strains from gastritis patients (32). What is the link between adherence and development of host pathology? First of all, increased adherence may lead to an increased bacterial burden. Second, studies in mice show that increased adherence does not necessarily lead to increased colonization density but to a closer contact between bacteria and gastric epithelial cells (34). A more intimate contact enhances the crosstalk between microorganism and host and may lead to activation of transcription factor NF-κ and host signal transduction pathways (20). This induces interleukin-8 (IL-8) production and inflammation, and finally, ulceration. This sequence of events is in agreement with data that show that increased Le^{x} expression in *H. pylori* is associated with increased neutrophil infiltration (36), and that strains isolated from patients with ulcers express an increased number of Lewis antigens as compared to strains from dyspeptic patients (76).

H. pylori

In summary, the mechanisms of *H. pylori* LPS phase variation are known in detail; knowledge of the biological role of Lewis antigens and phase variation therein is in its infancy, but a role in adhesion seems likely.

H. pylori

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